SUBSTITUTE FORM PTO-1390	U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER 06501-058001
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U.S. APPLICATION NO. (IF KNOWN) 09/529962
INTERNATIONAL APPLICATION NO. PCT/JP98/04772	INTERNATIONAL FILING DATE October 21, 1998	PRIORITY DATE CLAIMED October 22, 1997

TITLE OF INVENTION
METHOD FOR SCREENING FULL-LENGTH CDNA CLONES

APPLICANT(S) FOR DO/EO/US
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Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a SECOND or SUBQUENT submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ A translation of the International Application (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ A translation of amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other documents or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☐ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☐ Other items or information:
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I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1 10 on the date indicated above and is addressed to the Assistant Commissioner For Patents, Washington, D.C 20231

Samantha Bell
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U.S. APPLICATION NO. (IF KNOWN) 09/529962	INTERNATIONAL APPLICATION NO. PCT/JP98/04772	ATTORNEY'S DOCKET NUMBER 06501-058001	
17. ☒ The following fees are submitted:		CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5)):			
Search report has been prepared by the EPO or JPO $840		$840.00	
International preliminary examination fee paid to USPTO (37 CFR 1.482) .. $670			
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) $690			
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO $970			
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2) to (4) $96			
ENTER APPROPRIATE BASIC FEE AMOUNT		$840.00	
Surcharge of $130 for furnishing the oath or declaration later than ☐ 20 ☐ 30 mos. from the earliest claimed priority date (37 CFR 1.492(e)).		$0.00	

Claims	Number Filed	Number Extra	Rate		
Total Claims	7 - 20		x $18	$0.00	
Independent Claims	2 - 3		x $78	$0.00	
Multiple Dependent Claims(s) (if applicable)			+ $260	$0.00	
TOTAL OF ABOVE CALCULATIONS				$840.00	
Reduction by ½ for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28.)				$0.00	
SUBTOTAL				$840.00	
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TOTAL NATIONAL FEE				$840.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31).				$0.00	
TOTAL FEES ENCLOSED				$840.00	
				Amount to be refunded	
				Charged	

a. ☒ A check in the amount of $840.00 to cover the above fees is enclosed.
b. ☐ Please charge my Deposit Account No. 06-1050 in the amount of $0.00 to cover the above fees. A duplicate copy of this sheet is enclosed.
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NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b) must be filed and granted to restore the application to pending status.

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SPECIFICATION

METHOD FOR SCREENING FULL-LENGTH cDNA CLONES

Technical field

The present invention belongs to the field of genetic engineering, and relates to a method for screening full-length cDNA clones.

Background Art

Recently, genome projects targeting various animals, plants, and microorganisms have been in progress. Numerous genes have been isolated and their functions are under investigation. In order to efficiently analyze the functions of isolated genes, it is important to efficiently obtain cDNA clones capable of expressing complete proteins, that is, full-length cDNA clones.

The followings are known as methods for constructing a full length-enriched cDNA library: the oligo capping method in which an RNA linker is enzymatically bound to Cap of mRNA (Sugano & Maruyama, Proteins, Nucleic Acids and Enzymes, 38: 476-481, 1993, Suzuki & Sugano, Proteins, Nucleic Acids and Enzymes, 41: 603-607, 1996, M. Maruyama and S. Sugano, Gene, 138, 171-174, 1994); the modified oligo capping method developed by combining the oligo capping method with Okayama-Berg method (S. Kato et al., Gene, 150, 243-250, 1994, Kato & Sekine, Unexamined Published Japanese Patent Application (JP-A) NO. Hei 6-153953, published June 3, 1994); and the linker chemical-binding method in which a DNA linker is bound to Cap (N. Merenkova and D. M. Edwards, WO 96/34981 Nov. 7, 1996), the cap chemical modification method by biotin modification of Cap (P. Carninci et al., Genomics, 37, 327-336, 1996, P. Carninci et al., DNA Research, 4, 61-66, 1997). These are all methods to modify Cap of eukaryotic mRNA and to prepare a full length-enriched cDNA library. A known method for constructing a full length-enriched cDNA library by trapping Cap is the method using Cap-binding proteins derived from yeast or Hela cells for labeling a 5'-cap site (I. Edery et al., MCB, 15, 3363-3371, 1995). Also known is Cap Finder (Clontech) that is the Cap Switch oligonucleotide method in which the Cap Switch oligonucleotide is annealed by C-tailing the 5' end of a first strand cDNA.

A cDNA library constructed by these methods is rich in full-length cDNAs compared to that obtained by the conventional methods. However, incomplete-length clones are also contained to some extent. To efficiently analyze the functions of genes and to efficiently clone novel useful genes, development of methods for easily confirming whether each clone contained in a cDNA library is full-length or not has been desired.

Disclosure of the Invention

An objective of the present invention is to provide a method for efficiently screening full-length cDNA clones, and a method for constructing a full length-enriched cDNA library.

The present inventors have studied to achieve the above objective and contemplated efficiently screening full-length cDNAs from a cDNA library by the presence or absence of a translation initiation codon as an index based on the fact that a cDNA deficient in a certain 5'-region is likely to lack a translation initiation codon, whereas a full-length cDNA contains an initiation codon. Especifically, the inventors assumed that a full-length cDNA could be efficiently screened from a cDNA library constructed by a method for preparing a full length-enriched cDNA library. Specifically, the inventors thought that full-length cDNA clones could be efficiently isolated by constructing a cDNA library by a method for preparing a full length-enriched cDNA library, determining several hundreds of base pairs of a DNA nucleotide sequence from the 5' end, and analyzing the presence or absence of an initiation codon in this region to screen the clones containing initiation codons.

However, few programs for predicting an initiation site of cDNA have been developed (e.g., "A. G. Pedersen, Proceedings of fifth international conference on intelligent systems for molecular biology, p226-233, 1997, held in Halkidiki, Greece, June 21-26, 1997). Though some programs for exons prediction have been developed ("Gene Finder". V. V. Solovyev et al., Nucleic Acids Res., 22, 5156-5163, 1994, "Grail" Y. Xu et al., Genet-Eng-N-Y., 16, 241-253, 1994), an initiation site cannot be accurately determined relying solely on these programs.

The present inventors have developed a program for cDNA initiation codon prediction by themselves and determined nucleotide sequences of the 5'-region of clones contained in a cDNA library constructed by a method for preparing a full

length-enriched cDNA library to examine whether an initiation codon exists in this 5'-region using this software program.

More specifically, a full length-enriched cDNA library was constructed by the oligo capping method and nucleotide sequences of the 5'-regions of some clones contained in the cDNA library were determined. Based on the determined sequences, the clones were divided into known and novel ones through a database search. The presence or absence of an initiation codon and its location in the determined nucleotide sequences of the 5'-regions were judged using the initiation codon prediction program. For the known clones, whether the location of the initiation codon recognized by the initiation codon prediction program coincides with that of the initiation codon in databases is examined. Indeed, the presence or absence and location of the initiation codon in the known clones predicted by the program coincided with the information in the databases.

Thus, the software program developed by the present inventors can accurately recognize the presence or absence of an initiation codon and its location, and full-length cDNA clones can be efficiently screened by selecting the clones that are recognized to contain an initiation codon by the program from the cDNA library. Moreover, a cDNA library extremely rich in full-length cDNAs can be constructed by combining the screened clones.

The present invention relates to a method for screening full-length cDNA clones from a cDNA library and a method for constructing a full-length cDNA library by combining cDNA clones screened by the screening method. More specifically, it relates to:

(1) A method for isolating a full-length cDNA clone, the method comprising:

(a) determining a nucleotide sequence from the 5'-region of a cDNA clone contained in a cDNA library,

(b) determining the presence or absence of an initiation codon in the nucleotide sequence determined in (a) using an initiation codon prediction program, and

(c) selecting clones recognized as containing the initiation codon in (b);

(2) The method of (1), wherein the cDNA library is constructed by a method for preparing a full length-enriched cDNA library;

(3) The method of (1), wherein a cDNA library is constructed by a method

comprising a step of modifying Cap of mRNA;

(4) A method for constructing a full length cDNA library, the method comprising:

(a) determining a nucleotide sequence from the 5'-region of a cDNA clone contained in a cDNA library,

(b) determining the presence or absence of an initiation codon in the nucleotide sequence determined in (a) using an initiation codon prediction program,

(c) selecting clones recognized as containing the initiation codon in (b), and

(d) combining the clones selected in (c);

(5) The method of (4), wherein the cDNA library is prepared by a method for constructing a full length-enriched cDNA library;

(6) The method of (4), wherein the cDNA library is constructed by a method comprising a step of modifying Cap of mRNA; and

(7) A cDNA library obtainable by the method of (4).

The present invention is based on the inventors' findings that full-length cDNA clones can be efficiently isolated by analyzing nucleotide sequences of the 5-region of cDNAs in a cDNA library, specifically a full length-enriched cDNA library, by using a software program for accurately predicting a translation initiation codon, and a full length-enriched cDNA library can be constructed by combining the isolated cDNA clones. The method for screening full-length cDNA clones by the present invention comprises (a) determining a nucleotide sequence from the 5'-region of a cDNA clone contained in a cDNA library, (b) determining the presence or absence of an initiation codon in the determined nucleotide sequence using an initiation codon prediction program, and (c) selecting clones recognized as containing the initiation codon. The method for constructing a full-length cDNA library of the present invention comprises, in addition to above steps (a) to (c), step (d) of combining the screened clones.

In the method of the present invention, a "cDNA clone" whose nucleotide sequence of the 5'-region is to be determined is not particularly limited. Full-length cDNAs cannot be efficiently isolated from clones derived from a library not rich in full-length cDNAs, compared with clones derived from a full length-enriched cDNA library. Therefore, a cDNA clone is preferably derived from a library

constructed by the above-described methods for preparing a full length-enriched cDNA library, including, for example, the oligo capping method in which an RNA linker is enzymatically bound to Cap of mRNA (Sugano & Maruyama, Proteins, Nucleic Acids and Enzymes, 38: 476-481, 1993, Suzuki & Sugano, Proteins, Nucleic Acids and Enzymes, 41: 603-607, 1996, M. Maruyama and S. Sugano, Gene, 138, 171-174, 1994), the modified oligo capping method developed by combining the oligo capping method with Okayama-Berg method (S. Kato et al., Gene, 150, 243-250, 1994, Kato & Sekine, JP-A-Hei 6-153953, June 3, 1994), the linker chemical-binding method in which a DNA linker is chemically bound to Cap (N. Merenkova and D. M. Edwards, WO 96/34981 Nov. 7, 1996), the Cap chemical modification method in which Cap is modified with biotin (P. Carninci et al., Genomics, 37, 327-336, 1996, P. Carninci et al., DNA Research, 4, 61-66, 1997), the method using Cap binding proteins drived from yeast or Hela cells (I. Edery et al., MCB, 15, 3363-3371, 1995), or a library prepared by Cap Finder using Cap Switch oligonucleotide method.

A cDNA clone can be isolated from a cDNA library by standard methods described in, for example, J. Sambrook, E. F. Fritsch & T. Maniatis, Molecular Cloning, Second Edition, Cold Spring Harbor Laboratory Press, 1989.

A nucleotide sequence can be determined from the 5'-region of a clone by, for example, standard methods using DNA sequencing reagents and a DNA sequencer available from Applied Biosystems, etc. A whole nucleotide sequence of the clone dose not have to be determined, and determining about 1,000 nucleotides from the 5' end is sufficient. The high accuracy can be expected by determining about 500 nucleotides, even about 300 nucleotides.

An "initiation codon prediction program" used for analyzing a nucleotide sequence from the 5'-region of a clone is preferably the program developed by the present inventors as described in Example 1 below. The presence or absence of an initiation codon in a determined sequence is judged by a score deduced from the results of analysis with the program. A cDNA clone with a high score, recognized as containing an initiation codon in the determined sequence, is usually comprised of a full-length cDNA, while one with a low score, recognized as not containing an initiation codon in the determined sequence, contains an incomplete-length cDNA. Thus, a full-length cDNA can be efficiently isolated by screening a cDNA from a

cDNA library, judged as containing an initiation codon in the nucleotide sequence. Indeed, in one embodiment of the analysis with the program described in Example 1 below where a cDNA library with the full-length cDNA content of 51% was used to screen clones (the highest score was 0.94), the content of full-length clones among the screened clones was 71% when clones showing a score of 0.5 or higher were selected, 77% with a score of 0.70 or higher, 81% with a score of 0.80 or higher, and 85% with a score of 0.90 or higher. Therefore, full-length cDNA clones can be screened with a high accuracy by selecting clones with high scores using the program described in Example 1.

Moreover, a cDNA library re-constructed by combining clones selected by the method for screening full-length cDNA clones of the present invention is extremely rich in full-length cDNAs compared with the parent cDNA library used for screening clones. By expressing whole cDNAs capable of expressing proteins in the thus-obtained library, a system for efficiently analyzing gene functions containing a mixture of expressed proteins can be obtained. This system enables efficiently cloning useful genes.

Best Mode for Carrying out the Invention

The present invention is illustrated in detail below with reference to the following examples, but is not to be construed as being limited thereto.

Example 1. Preparation of a program for predicting a translation initiation codon of cDNA

The translation initiation codon prediction program of the present invention recognizes a putative authentic initiation codon among all ATGs contained in a given cDNA sequence fragment. The program predicts based on A) information on similarity of given regions (several tens to several hundreds base pairs) at both sides of a putative ATG to translational regions and B) information on similarity of regions near a putative ATG to those near an authentic initiation codon. Characteristics of sequences in a translational region and regions near an initiation codon are extracted beforehand by from information of numerous sequences whose translational and non-translational regions have been identified. The program predicts an initiation codon based on the information about the above

characteristics.

The linear discriminant analysis used in Gene Finder, a program for genomic exon prediction (Solovyev V. V., Salamov A. A., Lawrence C. B. Predicting internal exons by oligonucleotide composition and discriminant analysis of spliceable open reading frames. Nucleic. Acids Res, 1994, 22: 5156-63), was applied to optimize prediction. In the linear discriminant analysis, information on some characteristics derived from data is digitizied, weighted, and then culculated a score. Here, a score is converted into a probability of similarity to an initiation codon (the probability is a rate of correct answers obtained from data of sequences whose initiation codon has been identified). Specifically, a probability of similarity to an initiation codon of each ATG contained in a given cDNA sequence is output. Recognition as an initiation codon is determined whether a probability of similarity to an initiation codon is above a given threshold value or not. A threshold value is established depending on the plan of the following analyses, that is, depending on the extent of noises acceptable for the following analysis. For example, when 40% of noise is acceptable, a threshold value of 0.6 can be used. A parameter of weight is determined so as to maximize the prediction system using data of sequences whose initiation codon has been identified as a training datum. The above information of A) and B) were each embodied into the following three information and used as information about characteristics.

A) information on similarity of given regions (several tens to several hundreds base pairs) at both sides of a putative ATG to translational regions

1: a frequency of six nucleotide base letters contained in a sequence from ATG to a stop codon (within 300 bp downstream of ATG at longest)

2: discrepancy of the information on a frequency of six nucleotide base letters contained in 50 nucleotide bases upstream and downstream of ATG

3: an index of similarity to a signal peptide [a hydrophobicity index of the most hydrophobic eight amino acids letters among 30 amino acids (90 nucleotide bases) downstream of ATG]

B) <u>information on similarity of regions near a putative ATG to those near an authentic initiation codon</u>

1: information on a weighted matrix as using three nucleotide base letters in the region from 14 nucleotide bases upstream of ATG to 5 nucleotide

bases downstream of ATG as a unit

2) the presence or absence of other ATGs upstream of ATG in a same frame (the presence is 1 and the absence is 0)

3: a frequency of cytosine contained in the region from 36 bases upstream of ATG to 7 bases downstream of ATG.

Example 2: Preparation of cDNA by the oligo capping method and analysis thereof by the program for initiation codon prediction

A cDNA library was prepared by the oligo capping method and the plasmid DNA was extracted from each clone by the standard method. Specifically, mRNA was extracted from human placenta and human cultured cells (Tetratocarcinoma NT-2 and neuroblatoma SK-N-MC) by the method described in the reference (J. Sambrook, E. F., Fritsch & T. Maniatis, Molecular Cloning, Second Edition, Cold Spring Harbor Laboratory Press, 1989). An oligo cap linker (SEQ ID NO. 1) with an oligo dT adaptor primer (SEQ ID NO. 2) in the case of Tables 1 & 2, or with a random adaptor primer (SEQ ID NO. 3) in the case of Tables 3 & 4 were subjected to BAP treatment, TAP treatment, RNA ligation, synthesis of a first strand cDNA, and removal of RNA according to the methods described in the references (Suzuki & Sugano, Proteins, Nucleic Acids, and Enzymes, 41, 603-607, 1996, p606, Y. Suzuki et al., Gene, 200, 149-156, 1997). The first strand cDNA was then converted into the double-stranded DNA by PCR, digested with *SFI*I, and cloned into vectors, such as pME18SCG, pMFL etc. digested with *Dra*III in the determined direction (Sugano & Maruyama, Proteins, Nucleic Acids, and Enzymes, 38, 472-481, 1993, p480). The obtained DNA was subjected to the sequencing reaction using a DNA sequencing reagent (DyeTerminatoir Cycle Sequencing FS Ready Reaction Kit, PE Applied Biosystems) following the manual and sequenced with a DNA sequencer (ABIPRISM 377, PE Applied Biosystems). The DNA sequence of the 5'-region of each clone was analyzed once.

The presence or absence of an initiation codon in the DNA sequence of each clone was analyzed using the developed program for cDNA initiation codon prediction (ATGpr). In this analyzing program, the higher the score is, the higher the probability of being an initiation codon is. The maximum score is 0.94.

(1) Analysis of translation initiation codons in the clones whose open reading frames are known in database among cDNA prepared by the oligo capping method

Among the results for all analyzed clones, the result for the clones that are known to contain the initiation codon in the determined sequences in databases (F-NT2RP1000020, F-NT2RP1000025, F-NT2RP1000039, and F-NT2RP1000046) are shown in Table 1. F-NT2RP1000020 (880 bp) has 96% identity at nucleotide positions 88 to 690 to "human neuron-specific gamma-2 enolase" (GenBank accession No. M22349); F-NT2RP1000025 (645 bp), 97% homology at positions 29 to 641 to "human alpha-tubulin mRNA" (GenBank accession No. K00558); F-NT2RP1000039 (820 bp), 96% identity at positions 12 to 820 to "human mRNA for elongation factor 1 alpha subunit (EF-1 alpha) (GenBank accession No. X03558); and F-NT2Rp1000046 (788 bp), 97% identity at positions 3-788 to "human M2-type pyruvate kinase mRNA" (GenBank accession No. M23725). The sequences of the 5'-region in these clones are shown in SEQ ID Nos: 4, 5, 6, and 7.

Table 1

	F-NT2RP1000020		F-NT2RP1000025		F-NT2RP1000039		F-NT2RP1000046	
ATG No.	Location of ATG	ATGpr Score	Location of ATG	ATGpr Score	Location of ATG	ATGpr Score	Location of ATG	ATGpr Score
1	1	0.05	96	<0.94>	65	<0.90>	111	<0.94>
2	162	<0.84>	148	0.13	154	0.05	174	0.82
3	292	0.05	193	0.05	209	0.11	198	0.19
4	313	0.05	201	0.09	231	0.05	300	0.16
5	441	0.05	232	0.05	321	0.05	315	0.11

Note 1: <> means translation initiation codon

Note 2: Location of ATG means the nucleotide base position of ATG in the 5'-region of a DNA sequence.

ATG No. means the number of ATG from the 5'-region of a DNA sequence.

As show in Table 1, among the cDNA prepared by the oligo capping method, the full-length clones whose open reading frames are known in databases, containing initiation codons were accurately recognized by the initiation codon prediction program (ATGpr) (coincident with the initiation codons in databases).

(2) Analysis of initiation codons in the clones whose open reading frames are known in database among cDNA prepared by the oligo capping method

Among the results for the clones analyzed, the results for the clones whose initiation codon is known to absent in the determined sequence in databases (F-NT2RP1000013, F-NT2RP1000054, and F-NT2RP1000122) are shown in Table 2. F-NT2RP1000013 (608 bp) has 97% identity at positions 1 to 606 to "human nuclear matrix protein 55 (nmt55) mRNA" (GenBank accession No.U89867); F-NT2RP1000054 (869 bp), 96% identity at positions 1 to 869 to "human signal recognition particle (SRP54) mRNA" (GenBank accession No. U51920); and F-NT2RP1000122 (813 bp), 98% identity at positions 1 to 813 to *"H. sapiens* mRNA for 2-5A binding protein" (GenBank accession No. X76388). The sequences of the 5' region of these clones are shown in SEQ ID Nos: 8, 9, and 10.

Table 2

	F-NT2RP1000013		F-NT2RP1000054		F-NT2RP1000122	
ATG No.	Location of ATG	ATGpr Score	Location of ATG	ATGpr Score	Location of ATG	ATGpr Score
1	21	0.05	31	0.12	23	0.07
2	27	0.05	60	0.20	100	0.05
3	32	0.32	87	0.05	166	0.05
4	56	0.11	97	0.05	235	0.06
5	119	0.10	146	0.05	316	0.05
6	125	0.08	172	0.05	346	0.05
7	141	0.05	180	0.11	406	0.05
8	155	0.06	218	0.07	431	0.05
9	161	0.06	272	0.05	469	0.06
10	176	0.08	319	0.07	546	0.12
11	203	0.07	346	0.05	553	0.05
12	290	0.20	363	0.07	574	0.05
13	311	0.16	409	0.05		
14	314	0.12	480	0.07		

As shown in Table 2, among cDNA prepared by oligo capping method, the

initiation codon prediction program (ATGpr) did not recognize by mistake the initiation codons in incomplete-length cDNAs whose open reading frames are known in databases and which do not contain any initiation codons.

(3) Analysis of initiation codons in novel clones among the cDNA prepared by the oligo capping method

Among the results for analyzed clones, the results for novel clones that were predicted to contain initiation codons (F-ZRV6C1000408, F-ZRV6C1000454, F-ZRV6C1000466, F-ZRV6C1000615, and F-ZRV6C1000670) are shown in Table 3. The sequences of the 5' region of these clones are shown in SEQ ID Nos: 11, 12, 13, 14, 15.

Table 3

	F-ZRV6C1000408		F-ZRV6C1000454		F-ZRV6C1000466	
ATG No.	Location of ATG	ATGpr Score	Location of ATG	ATGpr Score	Location of ATG	ATGpr Score
1	85	<0.94>	5	0.05	162	<0.86>
2	208	0.22	107	<0.87>	182	0.05
3	386	0.05	153	0.05	207	0.08
4	518	0.11	201	0.08	244	0.05
5	545	0.05	211	0.05	262	0.05
6			236	0.07	303	0.11

(cont'd)

Table 3 (cont'd)

	F-ZRV6C1000615		F-ZRV6C1000670	
ATG No.	Location of ATG	ATGpr Score	Location of ATG	ATGpr Score
1	85	<0.94>	120	<0.94>
2	208	0.26	187	0.54
3	386	0.05	312	0.06
4	518	0.09	388	0.05
5	545	0.05	445	0.05

Note: <> means predicted initiation codon.

As shown in Table 3, the predicted initiation codons in F-ZRV6C1000408, F-ZRV6C1000454, F-ZRV6C1000466, F-ZRV6C1000615, and F-ZRV6C1000670 are "ATG" starting with "A" at positions 85, 107, 162, 85, and 120, respectively. Therefore, these clones were judged as full-length cDNA clones.

In addition, among the results for analyzed clones, the results for novel clones predicted as not containing initiation codons (F-ZRV6C1001410, F-ZRV6C1001197, and F-ZRV6C1001472) are shown in Table 4. The sequences of the 5' region of these clones are shown in SEQ ID Nos: 16, 17 and 18.

Table 4

	F-ZRV6C1001410		F-ZRV6C1001197		F-ZRV6C1001472	
ATG No.	Location of ATG	ATGpr Score	Location of ATG	ATGpr Score	Location of ATG	ATGpr Score
1	23	0.05	5	0.24	77	0.25
2	31	0.07	141	0.25	126	0.05
3	71	0.06	202	0.05	149	0.05
4	178	0.05	219	0.05	194	0.05
5	214	0.05	228	0.05	213	0.22
6					249	0.05
7					338	0.09
8					344	0.05
9					351	0.05
10					365	0.05

As shown in Table 4, F-ZRV6C1001410, F-ZRV6C1001197, and F-ZRV6C1001472 were recognized as not containing initiation codons. These clones were thus judged as incomplete-length clones.

Industrial Applicability

The present invention provides a method for efficiently selecting full-length cDNAs. Clones selected by the method of the present invention can express complete proteins. Therefore, the present invention enables efficiently analyzing the functions of isolated genes.

CLAIMS

1. A method for isolating a full-length cDNA clone, the method comprising:

(a) determining a nucleotide sequence from the 5'-region of a cDNA clone contained in a cDNA library;

(b) determining the presence or absence of an initiation codon in the nucleotide sequence determined in (a) using an initiation codon prediction program; and

(c) selecting clones recognized as containing the initiation codon in (b).

2. The method of claim 1, wherein the cDNA library is constructed by a method for preparing a full length-enriched cDNA library.

3. The method of claim 1, wherein a cDNA library is constructed by a method comprising a step of modifying Cap of mRNA.

4. A method for constructing a full length cDNA library, the method comprising:

(a) determining a nucleotide sequence from the 5'-region of a cDNA clone contained in a cDNA library;

(b) determining the presence or absence of an initiation codon in the nucleotide sequence determined in (a) using an initiation codon prediction program;

(c) selecting clones recognized as containing the initiation codon in (b); and

(d) combining the clones selected in (c).

5. The method of claim 4, wherein the cDNA library is prepared by a method for constructing a full length-enriched cDNA library.

6. The method of claim 4, wherein the cDNA library is constructed by a method comprising a step of modifying Cap of mRNA.

7. A cDNA library obtainable by the method of claim 4.

Abstract

A method for efficiently screening full-length cDNA clones, the method comprising determining a nucleotide sequence of the 5'-region of a clone contained in a cDNA library prepared by a method for constructing a full length-enriched cDNA library and examining the presence or absence and the location of a translation initiation codon in the 5'-region using an originally developed program for predicting initiation codons in cDNA. This originally developed program accurately predicts the presence or absence and the location of initiation codons and efficiently screens full-length cDNA clones by selecting clones judged as containing an initiation codon from a cDNA library. Moreover, a cDNA library extremely rich in full-length cDNAs can be constructed by combing the selected clones.

SEQUENCE LISTING

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<120> Method for screening full-length cDNA clones

<130> H1-806PCT

<150> JP 09-289982
<151> 1997-10-22

<160> 18

<170> PatentIn version 2.0

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CATATTTTAG ACTGAAAAAA TTTGCTGTTG CTGAATCTGA TTGTTATTTAN CANTTGCCT 660

TGAAATA 667

Attorney's Docket No.: 06501-058001
Client's Ref. No.: H1-806PCT-US

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled METHOD FOR SCREENING FULL-LENGTH cDNA CLONES, the specification of which:

[] is attached hereto.
[X] was filed on April 20, 2000 as Application Serial No. 09/529,962 and was amended on _____.
[] was described and claimed in PCT International Application No. _____ filed on _____ and as amended under PCT Article 19 on _____.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose all information I know to be material to patentability in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

Country	Application No.	Filing Date	Priority Claimed
Japan	9/289982	October 22, 1997	[X] Yes [] No
PCT	PCT/JP98/04772	October 21, 1998	[X] Yes [] No

I hereby appoint the following attorneys and/or agents to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

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Inventor's Signature: Toshio Ota Date: 11/17/00

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Inventor's Signature: Takao Isogai Date: 10/3/00

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Chiba 292-0833 Japan JPX

Citizenship: Japan

Post Office Address: 3-9-17-606, Kaibuchi, Kisarazu-shi
Chiba 292-0833 Japan

ATTORNEY DOCKET NO. 06501-058001

Applicant or Patentee: Toshio Ota et al
Serial or Patent No.: 09/529,962
Filed or Issued: April 20, 2000
For: METHOD FOR SCREENING FULL-LENGTH cDNA CLONES

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) and 1.27(c)) — SMALL BUSINESS CONCERN

I hereby declare that I am

[] the owner of the small business concern identified below:
[X] an official of the small business concern empowered to act on behalf of the concern identified below:

Name of Small Business Concern: HELIX RESEARCH INSTITUTE
Address of Small Business Concern: 1532-3, Yana, Kisarazu-shi
CHIBA 292-0812 JAPAN

I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 13 CFR 121.12, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees to the United States Patent and Trademark Office, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention, entitled METHOD FOR SCREENING FULL-LENGTH CDNA CLONES by inventor(s) TOSHIO OTA, TETSUO NISHIKAWA, ASAF SALAMOV AND TAKAO ISOGAI described in:

[] the specification filed herewith.
[X] application serial no. 09/529,962, filed April 20, 2000.
[] patent no. _, issued _.

If the rights held by the above identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 CFR 1.9(c) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d), or a nonprofit organization under 37 CFR 1.9(e). *NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

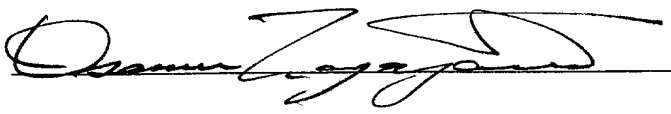
Full Name: _____
Address: _____

[] INDIVIDUAL [] SMALL BUSINESS CONCERN [] NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status when any new rule 53 application is filed or prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent on which this verified statement is directed.

Name:	Osamu Nagayama
Title:	Chief Executive Officer
Address:	1532-3, Yana, Kisarazu-shi CHIBA 292-0812 JAPAN

Signature:  **Date:** November 30, 2000